Review article

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A Brief Review on Dried Blood Spots Applications in Drug Development

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Abstract

Dried blood spots (DBS) refers to a blood sampling technique where small volumes of blood are spotted on filter paper, dried and analyzed. The DBS sampling technique was first developed to screen newborn babies for the genetic metabolic disorder phenylketonuria. Recently, this technique has been applied to pharmacokinetic, therapeutic drug monitoring and toxico-kinetic studies to reduce expenses during drug development. The DBS sampling technique has several advantages over conventional blood or plasma sampling in that it is less invasive, relatively painless, less blood volumes, utilizes simple storage methods depending on analyte stability, minimizes shipping expenses, offers convenient sampling and the reduces risk of blood borne pathogens. DBS reported for biological molecule measurement and detection of various drugs like Acetaminophen, Fluconazole, Metformin and Valsartan. This method of blood collection is particularly suited for developing countries where cost cutting is important. DBS method requires a hole-punch from a core sample, extraction with a solvent and subsequent liquid chromatography-mass spectroscopy (LC-MS), Laser absorption electrospray ionization (LAESI)-MS or immunoassay analysis. Disadvantages include requirements for assay development and validation as well as the relatively small volumes of sample. Present work describes the analytical concepts of DBS techniques along with DBS sample collection, processing and storage.

Keywords: Dried blood spots, Therapeutic drug monitoring, LC-MS, LAESI-MS, Immunoassay analysis

INTRODUCTION

Dried blood spots (DBS) refers to a blood sampling technique where small volumes of blood are spotted on an appropriate filter paper, dried, and taken to the laboratory for analysis. The technique is well established in clinical labs for applications such as neonatal screening for inborn diseases, but has recently experienced a surge of interest in the context of drug development, i.e. toxicokinetic, pharmacokinetic studies and therapeutic drug monitoring. Dried blood spot technology has many advantages over the conventional plasma sampling.

DBS HISTORY

In 1963, Guthrie et al. introduced DBS in the context of neonatal screening of phenylketonuria. This approach has had a massive success for many years, and heel pricks of neonates with sampling on DBS cards are practiced up to today in many countries worldwide. FDA has already registered two sources of filter paper for blood collection as Class II Medical Devices (21 CFR §862.1675) based on sustained compliance with the performance parameters specified in the Clinical and Laboratory Standards Institute (CLSI) LA4-A5 approved standard and these have been tested in the context of neonatal screening. [1] Drug metabolism (DM), pharmacokinetic (PK) and toxicokinetic (TK) studies provide crucial insight into how drug candidates behave in the body and therefore critical steps in drug development. These types of analysis traditionally require large volumes of blood (generally 100-500µl per subject per time point) to provide sufficient plasma volume for quantitative bioanalysis. However, only a limited blood volume can be drawn from each animal the number of serial samples is restricted and composite sampling must be used. This use results in lower quality PK data and an increase in the number of animals

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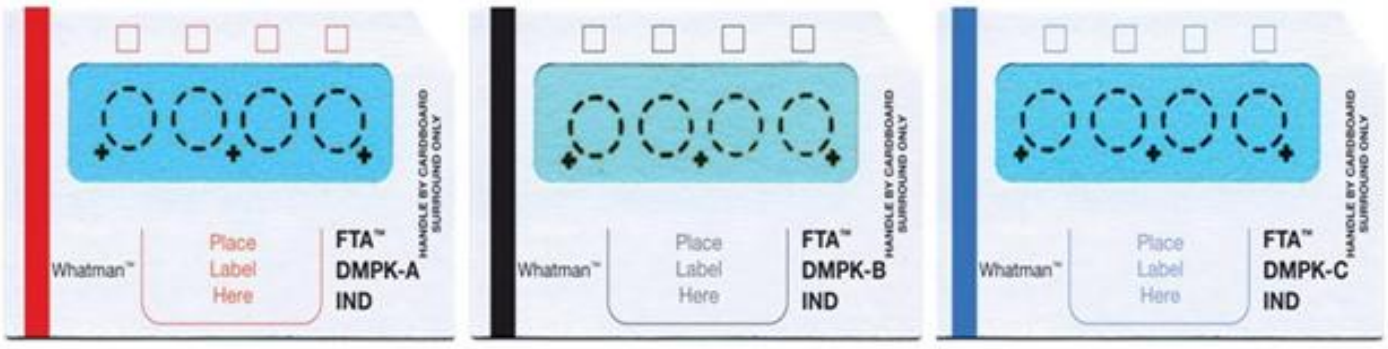
required. The large volumes also make it difficult to conduct studies in juvenile subjects. Additionally, plasma needs to be isolated from whole blood and prepared for bioanalysis using solid phase extraction, liquid-liquid extraction or protein precipitation. This time-consuming process limits throughput and consequently low samples can be tested. There are practical challenges with shipping and storing blood samples that require controlled handling and frozen transportation and storage. The dried blood spot (DBS) method is an alternative technique that overcomes these drawbacks.

FTA®DMPK CARDS

DBS micro volume sampling using specialized Whatman media from GE Healthcare has been shown to be precise and accurate for a variety of compounds from different structural classes with acceptable inter- and intra-assay variability. It is now being routinely employed in PK/TK studies.

Figure 1. FTA®DMPK-A and FTA DMPK-B



FTA DMPK Card Selection

FTA DMPK Card choice is depends on handling and performance criteria. Handling requirements may be influenced by operational or safety factors while performance depends on analyte chemical structure, extraction solvent and analysis workflow. There are 3 types of FTA DMPK Card are available (Figure 1).

FTA DMPK-A

a) Blood spots dry within 2 h
b) Blood spot area is ~20% smaller than DMPK-B or DMPK-C cards
c) Protein denaturing activity will inactivate endogenous enzymes
d) Cell lysis releases endogenous cellular materials onto card
e) Stabilization of DNA allows resampling of blood spot for pharmacogenomics
f) Impregnated chemicals may interfere with mass spectrometry detection e.g. ion suppression

FTA DMPK-B

a) Blood spots dry within 2 h
b) Protein denaturing activity will inactivate endogenous enzymes
c) Cell lysis releases endogenous cellular materials onto card
d) Stabilization of DNA allows resampling of blood spot for pharmacogenomics
e) Impregnated chemicals may interfere with mass spectrometry detection e.g. ion suppression

FTA DMPK-C

a) Blood spots dry within 2 h
b) No impregnated chemicals to interfere with analysis
c) Proteins are not denatured so cards may be better suited for protein based biomolecules

FTA®DMPK-A and FTA DMPK-B cards lyses cells and denature proteins on contact. Samples can be shipped

and stored at ambient temperature and long-term stability has been demonstrated for analytes and metabolites sensitive to plasma enzymes. [2]

PROCEDURE: SPOT EXTRACT ANALYZE

Apply blood to card and let dry (figure 2, 3). Ship and store as needed at ambient temperature.

Figure 2. Blood sample application

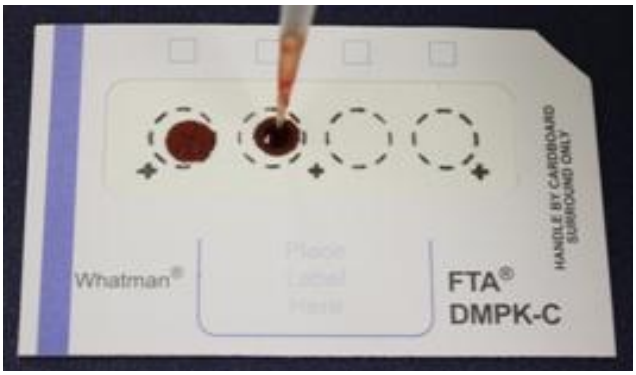
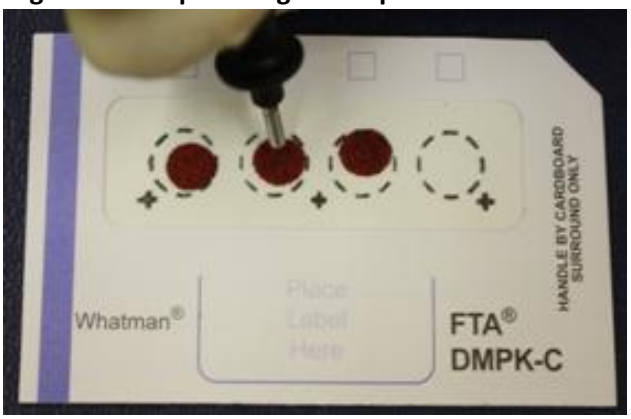


Figure 3. Disk punching of sample



SIMPLE AND SAFE PROCESSING

(i) The 3-step DBS procedure is much more straightforward than the cumbersome centrifugation, isolation and clean-up of plasma.

(ii) On-substrate clean-up has the potential to convey greater analyte stability, especially for enzyme-sensitive compounds.

For quantitative analysis of various small molecules, solution analysed by (a) HPLC-MS/MS (b) Tandem mass spectrometry (MS/MS) (c) DBS-LC-MS/MS (d) LAESI-MS (e) Immunoassay analysis.

Laser ablation electrospray ionization (LAESI) is ionization method for the identification of metabolites and biomolecules in biofluids or from surfaces with little or no sample preparation. This report describes another novel application for LAESI-MS technology in which DBS cards are directly analysed. [3]

BENEFITS OF USING THE DBS SAMPLING TECHNIQUE

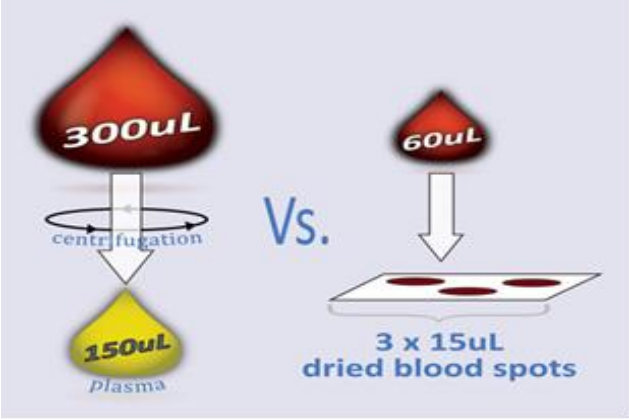
DBS sampling can offer enormous advantages over liquid blood or plasma in both preclinical and clinical studies. These advantages include, but are not limited to, a significant reduction in the volume of blood collected, a simplified process that does not require the need to centrifuge, sub-aliquot, freeze, and defrost samples (all of which can introduce errors in the analysis), improved safety with handling, shipping, and storage at room temperature, improved data quality, improved compound stability for drugs and their metabolites, and considerable cost savings.

PRECLINICAL ADVANTAGES

The use of DBS in preclinical studies results in a fivefold reduction in the volume of blood collected, which has a significant impact on animal studies and data quality (figure 4). The number of rodents needed for each study can be reduced by up to 75%, and because fewer animals are needed, the quantity of compound needed for testing is also greatly reduced. The quantity of compound required for animal studies is very important in the early stages of drug development when the synthesis of the compound has not yet been optimized and is costly, time-consuming, and difficult to achieve. DBS sampling also contributes to the generation of higher quality data in pre-clinical studies because more time points can be added without the need for additional rodents and the technology allows for serial pharmacokinetic (PK) profiling. Serial PK profiling eliminates the variability between animals

observed when using composite profiling and greatly improves the quality of the data. In addition, DBS allows for pre-clinical juvenile toxicology studies to be conducted in small animals where the availability of blood has always been a problem. These studies are always necessary and required by regulatory agencies in support of clinical pediatric studies. It is both ethically and medically not justifiable to draw large volumes of blood from neonates or infants.

Figure 4. Blood volume requirement



CLINICAL ADVANTAGES

As drug development programs progress into the clinic, the continued use of DBS provides additional benefits and cost savings. The simplified, less invasive blood sampling (finger/heel prick) is much more patient friendly than blood draws, especially for pediatric studies and in critically ill patients. The shipping, handling, and storage costs are also greatly reduced because DBS is safe and can be stored at room temperature. Unlike liquid blood or plasma, DBS does not need to be handled as a biohazard since pathogens like HIV and hepatitis B and other infectious pathogens are inactivated. Biohazardous samples are not only expensive to ship, but special training and licensing are also required. In addition, certain countries will not let blood samples be sent out of the country because they are biohazardous. The use of DBS can overcome these barriers. Sample handling is much easier using DBS because the sample does not need to be centrifuged for plasma and then transferred to secondary tubes for freezing. Finally, refrigeration is not needed during transport or for storage as the DBS can be stored at room temperature. Normally, specialized couriers are needed to ship samples in dry ice, and for large Phase II/III clinical trials that require thousands of samples to be shipped from different sites, this process can be very expensive using liquid blood. The reduced amount of specialized equipment at clinical sites (refrigerated centrifuge, monitored freezers) also makes DBS technology extremely valuable when conducting clinical studies in emerging countries. Toxicokinetic study designs in rodents are often based on a main group of animals that is assessed for toxicological effects of the administered drug, and a satellite group of animals that is used for pharmacokinetic assessment. The low volume DBS strategy can mean that in many cases the blood sampling is done directly from the main group of animals, without impact on their well-being and on any potential toxicological observations. Without the need for satellite group animals, this approach reduces the number of test animals in TK studies, which is an obvious ethical and cost-related advantage. Furthermore, as the DBS approach consumes less blood per time point than a traditional blood to plasma strategy, it is possible to collect pharmacokinetic profiles from single animals, rather than having to draw conclusions from composite profiles from different animals. The consequence is that better quality TK data is obtained. In terms of logistics, DBS offers a huge simplification. The dried filter papers can be shipped at ambient conditions in a simple envelope/pouch made up of plastic and preferably with some desiccant material.

DISADVANTAGES

Factors that will affect the bioanalysis are impact of blood volume spotted, impact of spotting with capillary or pipette, optimization of the extraction, the selection

of the type of card and DBS-specific questions that need to be looked at on a case by case basis. [4] Standard clinical assays (e.g., glycosylated hemoglobin, total cholesterol) are performed on automated, high-throughput analyzers using serum or plasma samples and offers increased speed and reduced costs of analysis but currently DBS samples are relatively having higher cost. DBS samples are a nonstandard diagnostic substance and DBS results may not directly comparable with serum or plasma results. DBS method requires assay development for samples and it may constrain flexibility for future biomarker measurement. The relatively small quantity of sample is collected in DBS method may become a limitation for some analytes that require large volumes of blood before more-sensitive protocols become available during initial research stage. [5]

DBS APPLICATIONS

Based on the advantages mentioned above, it is likely that DBS will find its place in the preclinical and clinical drug development. There are published accounts of such studies, providing proof of concept, and it is clear from presentations on DBS sessions during meetings by DIA, AAPS, EBF and other organizations during 2009 and 2010 that a significant number of innovator pharmaceutical companies are evaluating the technique in their own drug development programs. GSK has been spearheading the recent interest in DBS for drug development applications. As early adopters, they use DBS as the recommended analytical approach for the assessment of PK/TK data for all new oral small molecule drug candidates, which have previously demonstrated a successful bioanalytical validation. [5] As the benefits of DBS are not restrained to preclinical studies, a DBS strategy can be considered for clinical phase I and IIa studies as well. Another interesting area is therapeutic drug monitoring (TDM), where circulating drug concentrations need to be monitored, typically for drugs with a narrow therapeutic window and/or large inter-subject variability. This area often involves clinical laboratories, and it is interesting to see that DBS has been used longer in TDM than in TK and PK studies. Examples include the monitoring of Metformin in diabetic patients, which reportedly has been done with DBS or the immunosuppressant everolimus in transplant patients. Eurofins Global Central Laboratory has performed therapeutic drug monitoring based on DBS for combined LC/MS-MS analysis of everolimus, tacrolimus and cyclosporin A. [7, 8] Applications of DMPK Cards are given in table 1 as follows. [2] Dried blood spot protocols developed for several analytes are shown in table 2 as follows.

Table 1. Applications of DMPK Cards

Drug	Company
Acetaminophen	GSK
Acetyl salicylic acid (aspirin)	Tandem Labs
Amiodarone	Agilent
Bosentan	Inovalab A.G./Actelion
Caffeine	GSK
Cyclosporin A	Quality Assurance
Diazepam	PPD/Seventh Wave
Desipramine	MPI Research
Fluconazole	Pfizer
Flurbiprofen	GSK
Metoprolol	Covance
Metformin	PPD/Seventh Wave
Naproxen	Algorithme
Nifedipine	GSK
O-desmethylmetoprolol	Covance
Omeprazole	GSK
Oseltamivir (Tami flu)	Roche
Paracetamol	GSK
Procaine	Tandem Labs
Riluzole	Tandem Labs
Tacrolimus	PPD/Seventh Wave
Valsartan	Toray Pharmaceuticals
Zatebradine	GSK

FUTURE DEVELOPMENTS

While manually punching and extracting is acceptable for small sample numbers, this is a laborious step that calls for automation. Various technological options are available or under development. Semi-automated punch robots are available that drop the punched material in a 96-well plate format. From there, extraction can be performed. An approach that cuts out the punching step altogether is directly interfacing the DBS filter paper to the LC/MS-MS, i.e. extracting on-line. Various attempts are made in this direction, including the use of a TLC extraction device (Camag) that elutes the spot; with the outlet interfaced directly to the MS. Alternative approaches are likely to be needed for interfacing solutions. It is likely that these will become available in the not too distant future.

Table 2. Dried blood spot protocols developed for several analytes [9-13]

Analyte	Method	LDL
Androstenedione	Radioimmunoassay (RIA)	0.012 ng/mL
Cortisol	Radioimmunoassay (RIA)	0.46 ug/dL
C-reactive protein	Enzyme-linked immunosorbent assay (ELISA)	0.19 mg/L
Estradiol	Radioimmunoassay (RIA)	2 pg/mL
Glucose	Enzymatic	0.26 mmol/L
Insulin	Radioimmunoassay (RIA)	5.9 pmol/L
Lipoprotein	Enzyme-linked immunosorbent assay (ELISA)	22 mg/L
Progesterone	Radioimmunoassay (RIA)	0.015 ng/mL
Testosterone	Radioimmunoassay (RIA)	0.015 ng/mL

LDL: Lower detection limit

CONCLUSION

DBS as a blood sampling technique for applications in drug development is a recent phenomenon, spurred by financial and ethical motivations. It is remarkable that the power of modern analytical technology has allowed an old sampling technique to become prominent in a short period of time. In view of its advantages compared to traditional plasma-based strategies, it can be expected that DBS is here to stay, certainly if DBS-based NDAs or submissions pass regulatory scrutiny.

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